July 31, 1950.

Dr. C. B. van Niel,
Hopkins Marine Station,
Pacific Grove, California.

Dear Dr. van Niel:

Forthwith, but under seprate cover, I am sending two cultures from Escherichia coli, K-12, for recombination experiments:

58-161	B-M-	(biotin, methionine)
W-1177	T-L-B_1- Lac- Mal-Xyl-Gal-Ara-Mtl-	V_1^r S^r
	(threonine, leucine, thiamin lactose, maltose, d-xylose, l-arabinose, mannitol phage T1 streptomycin)	

As we discussed, the easiest characters to classify among prototrophs would be Lac and Mal, using EMB agar with 1% and 1½% sugar respectively. The other characters can be ighored. The easiest technique is as follows:

Inoculate separate cultures from slant into Pennassay broth (or any other nutrient broth without too much sugar), incubate overnight at 35-37 without shaking or aeration. Wash cells, mix equal aliquots of concentrated suspensions and spread on thiamin supplemented plates of minimal agar, about 10^8 - 5 x 10^8 per plate. Use thick plates (25 ml agar/10 cm diameter plate). Incubate at 30-37 for 48-72 hours. Pick prototrophs, etc.

EMB plates should be incubated at 37 about 20 hours. Especially with EMB Maltose, Mal⁄ tends to fade after a time.

If you wish to make up a more complicated experiment, you can also score S^r on the EMB plates by streaking first a solution of streptomycin, 10^5 u/ml, and cross-streaking the bacterial suspensions after the streptomycin has dried into the agar. S is very closely linked to Mal.

I would appreciate learning how this goes. Let me know if I can help in any way.

Sincerely,

Joshua Lederberg